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SET Nuclear Oncogene Associates with Microcephalin/MCPH1 and Regulates Chromosome Condensation*

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Primary microcephaly is an autosomal recessive disorder characterized by marked reduction in human brain size. Microcephalin (*MCPH1*), one of the genes mutated in primary microcephaly, plays an important role in DNA damage checkpoint control and mitotic entry. Additionally, MCPH1 ensures the proper temporal activation of chromosome condensation during mitosis, by acting as a negative regulator of the condensin II complex. We previously found that deletion of the of the MCPH1 N terminus leads to the premature chromosome condensation (PCC) phenotype. In the present study, we unexpectedly observed that a truncated form of MCPH1 appears to be expressed in MCPH1$^{S25X/S25X}$ patient cells. This likely results from utilization of an alternative translational start codon, which would produce a mutant MCPH1 protein with a small deletion of its N-terminal BRCT domain. Furthermore, missense mutations in the *MCPH1* cluster at its N terminus, suggesting that intact function of this BRCT protein-interaction domain is required both for coordinating chromosome condensation and human brain development. Subsequently, we identified the SET nuclear oncogene as a direct binding partner of the MCPH1 N-terminal BRCT domain. Cells with SET knockdown exhibited abnormal condensed chromosomes similar to those observed in MCPH1-deficient mouse embryonic fibroblasts. Condensin II knockdown rescued the abnormal chromosome condensation phenotype in SET-depleted cells. In addition, *MCPH1* V50G/I51V missense mutations, impair binding to SET and fail to fully rescue the abnormal chromosome condensation phenotype in Mcph1$^{-/-}$ mouse embryonic fibroblasts. Collectively, our findings suggest that SET is an important regulator of chromosome condensation/decondensation and that disruption of the MCPH1-SET interaction might be important for the pathogenesis of primary microcephaly.

Primary microcephaly is an autosomal recessive neurodevelopmental disorder characterized by markedly reduced brain size and mild-to-moderate mental retardation (1). Genetic studies have identified microcephalin (MCPH1) as one of seven genes that can cause primary microcephaly (OMIM Database ID 251200). The *MCPH1* gene encodes an 835-amino acid protein that contains three BRCA1 C-terminal (BRCT)[3] domains (2). This protein has also been identified as a transcriptional repressor of telomerase reverse transcriptase (BRIT; BRCT-repeat inhibitor of hTERT expression) (3) and has a role in DNA damage-induced S and G_2/M checkpoints. Ionizing radiation-induced foci of MCPH1 co-localize with MDC1 and phosphorylated H2AX (4–6), this colocalization is likely to be mediated by a direct interaction between the C-terminal tandem BRCT domains of MCPH1 and phosphorylated H2AX. Conversely, the N-terminal BRCT domain of MCPH1 is required for the centrosomal localization of vertebrate MCPH1 and for the regulation of chromosome condensation to ensure coordinate mitotic entry (7, 8).

A unique feature of cells derived from patients with *MCPH1* mutations is the premature chromosome condensation (PCC) phenotype (9, 10), in which mitotic chromosomes condense early in an otherwise unperturbed cell cycle. In routine diagnostic cytogenetic preparations, this is reflected by a large number of prophase-like cells being observed (10–15% of total cell number). In such cells, chromosomes are highly condensed to a level similar to that normally observed at metaphase, whereas the nuclear envelope still remains intact. Furthermore, MCPH1 cell metaphases exhibit shortened chromosomes with highly compacted and condensed DNA. This cellular phenotype likely results from premature onset of chromosome condensation mediated by the condensin II complex as depletion of condensin II rescues the PCC phenotype in patient cells (9, 10). Significantly, we recently reported that MCPH1 stably associates with condensin II (8). However, we unexpectedly found that reconstitution of WT MCPH1 or various mutants of MCPH1 into Mcph1$^{-/-}$ mouse embryonic fibroblasts (MEFs) revealed that the N terminus, but not the middle condensin II-binding

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[3] The abbreviations used are: BRCT, BRCA1 C-terminal; LCL, lymphoblastoid cell line; MCPH1, microcephalin; MEF, mouse embryonic fibroblast; PCC, premature chromosome condensation; SFB, S-protein, FLAG, and streptavidin-binding peptide; TAP, tandem affinity purification.

domain, of MCPH1 is responsible for preventing the PCC phenotype in MCPH1-deficient cells (8). The mechanism by which deletion of the MCPH1 N terminus causes PCC remains unknown.

In the current study, we sought to identify MCPH1-interacting partners involved in the regulation of chromosome condensation. Using a tandem affinity purification (TAP) scheme, we identified SET protein as a candidate that associates with the N terminus of MCPH1. We found that SET plays a role in MCPH1-mediated chromosome condensation/decondensation. Thus, we propose that SET and MCPH1 act together to regulate chromosome condensation and ensure faithful mitotic entry.

EXPERIMENTAL PROCEDURES

Cell Lines, Plasmids, and Antibodies—Lymphoblastoid cell lines with the MCPH1 S25*X* mutations from patients with primary microcephaly have been reported previously (5). The CV1744 lymphoblastoid cell line contains a homozygous deletion of the promoter and exons 1–8 of MCPH1.[4] H1299 and 293T cells were purchased from American Tissue Type Culture Collection (Manassas, VA) and maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C in 5% CO_2 (v/v). Mcph1 knock-out and WT MEFs were cultured in DMEM supplemented with 10% FBS, 1% non-essential amino acids, and 1% penicillin/streptomycin.

Deletion mutants of MCPH1 were generated as described previously (8). Site-directed mutagenesis of MCPH1 was performed according to standard procedures to obtain V50G/I51V using the primers 5′-AGGTTTCAAAAACTTTTAACAAACAAGTAACTCACGGTGTCTTCAAAGATGGCTACCAGAGCACTTGGGACAAAG-3′ and 5′-CTTTCTCCCAAGTGCTCTGGTAGCCATCTTTGAAACACCGTGAGTTACTTGTTTGTTAAAAGTTTTTGAAACCT-3′. SET cDNA was purchased from Open Biosystems (Huntsville, AL). All of these constructs were cloned into a Gateway-compatible destination vector harboring an N-terminal SFB tag (S-protein, a FLAG epitope, and a streptavidin-binding peptide) (11).

The MCPH1 polyclonal antibody was generated by immunizing rabbits with maltose-binding protein fusions of the middle and C-terminal BRCT domains of MCPH1. The antibody was purified using the Amino Link Immobilization kit (Pierce). SET polyclonal antibody (SC-25564) was obtained from Santa Cruz Biotechnology. hCAP-D3 polyclonal antibody was purchased from Bethyl Laboratories, and anti-FLAG M2 antibody was purchased from Sigma-Aldrich. S-protein beads and streptavidin-conjugated beads were purchased from Novagen and GE Healthcare, respectively. Cells were irradiated (10-Gy) using a Cs137 source from J. L. Shepherd.

Plasmid and siRNA Transfection—Plasmid transfection and siRNA transfection were carried out using Lipofectamine 2000 and Oligofectamine, respectively (Invitrogen), according to the manufacturer's instructions. The coding strand for the control siRNA was UCCAGUGAAUCCUUGAGGU UU and for the hCAP-D3 siRNA was CUGGAUUUCACAGAGACUGTTUU. Two sets of siRNAs were used in murine cell lines and human cell lines for SET down-regulation. The coding strands were CGCUCAAGUCAA ACGCAAAUU and GAGUUGACAUUCUG UUGUA for murine cells and AGGAGAAGAUGACUAAAUAUU and GAAGUCCACCGAAUCAAAUU for human cells. All siRNA were purchased from Dharmacon.

Reconstitution of WT or mutant MCPH1 into Mcph1$^{-/-}$ MEFs was performed by retroviral infection. For viral particle packaging, BOSC23 cells were transfected with the pCL-Ampho and expression constructs. Viral supernatant was collected 48 h after transfection and used for infection.

Tandem Affinity Purification and Mass Spectrometry Analysis—293T cells were transfected with plasmid encoding an SFB-tagged N-terminal fragment of MCPH1 (N-BRCT; residues 1–130). Stable cell lines were selected using 2 μg/ml puromycin and confirmed by Western blotting. TAP was performed as described previously (8), with minor modifications. Briefly, 293T cell lines stably expressing the MCPH1 N terminus were lysed with NETN buffer (100 mM NaCl, 1 mM EDTA, 20 mM Tris-HCl at pH 8.0 and 0.5% Nonidet P-40) and rocked for 20 min at 4 °C. Crude lysates were cleared by centrifugation at 14,000 rpm at 4 °C for 20 min. The supernatants were incubated with 300 μl of streptavidin-conjugated beads. The precipitated complexes were washed with NETN buffer, and then the bound proteins were eluted with 1.5 ml of NETN buffer containing 2 mg/ml biotin (Sigma-Aldrich). The eluted supernatant was incubated overnight with 50 μl of S-protein beads. The precipitated complexes were washed three times with NETN buffer. Protein mixtures were eluted and analyzed by LC-MS/MS at the Taplin Mass Spectrometry Facility at Harvard Medical School (Boston, MA).

Co-precipitation and GST Pulldown Assay—For co-precipitation studies, constructs encoding SFB-tagged MCPH1 and its mutants were transiently transfected into 293T cells using Lipofectamine 2000. Cells were then lysed in NETN buffer, and lysates were cleared by centrifugation at 14,000 rpm at 4 °C for 20 min. The lysates were then incubated with streptavidin-conjugated beads for 2 h at 4 °C. The resulting precipitated complexes were washed with NETN, boiled in sample buffer, subjected to SDS-PAGE, and immunoblotted with antibodies as indicated.

cDNAs encoding SET, WT-MCPH1, or MCPH1 mutants were subcloned into pDEST15 vector for the expression of GST fusion proteins in *Escherichia coli*. All GST fusion proteins were purified and immobilized on glutathione-Sepharose (GE Healthcare) according to the manufacturer's instructions. For the GST pulldown assay, 293T lysates containing SFB-tagged SET, WT-MCPH1, or MCPH1 mutants were incubated with beads coated with GST or GST fusion proteins for 2 h at 4 °C. The beads were washed extensively, and associated proteins were eluted by boiling in sample buffer. The samples were then separated by SDS-PAGE and immunoblotted with anti-FLAG antibody.

Metaphase Spreads—Metaphase chromosome spreads were performed as described previously (12). Briefly, cells were treated with colcemid for 4 h and then harvested. The cells were then treated with 0.075 M KCl at 37 °C for 20 min, fixed in fresh methanol/acetic acid (3:1) solution, and dropped

[4] L. Bicknell and A. Jackson, unpublished data.

onto coverslips. Cells were allowed to air dry on the coverslips, stained with Giemsa, and visualized using a Nikon Eclipse E800 microscope. Quantification and scoring were carried out as described previously (8).

Immunoprecipitation (IP) Western Blotting—For IP Western blotting, patient cells were lysed with NETN buffer on ice for 20 min and cleared by centrifugation at 14,000 rpm for 20 min. MCPH1 antibody was added to the lysates and rocked for 1 h. Protein A-Sepharose beads were then added and incubated for overnight at 4 °C. The immunocomplexes were washed for three times and subjected to SDS-PAGE. Membranes were blocked with 5% nonfat milk in Tris-buffered saline Tween 20 solution and then probed with antibodies as indicated.

Genotyping and Mutation Detection—Genomic DNA was extracted by standard methods. PCR amplification of coding exons of MCPH1 was performed using previously published primers and conditions (1). Purified PCR amplification products were sequenced using dye-terminator chemistry and electrophoresed on an ABI 3730 capillary sequencer (Applied Biosystems). Mutation analysis was performed using Mutation Surveyor software (Softgenetics). Anonymised control samples were screened by sequencing. Mutation identification was performed as part of a study approved by the Scottish Multicenter Research Ethics Committee (04:MRE00/19).

Statistical Analyses—Data are presented as mean ± S.E., and all statistical analyses were performed using GraphPad Prism software. $p < 0.05$ was considered statistically significant.

RESULTS

Disruption of N-terminal BRCT Domain Causes Primary Microcephaly and Premature Chromosome Condensation without Perturbing Ionizing Radiation-induced Foci Formation—The hallmark of cells obtained from human patients with *MCPH1* mutations or Mcph1$^{-/-}$ MEFs is the presence of prematurely condensed chromosomes. We therefore further characterized the MCPH1$^{S25X/S25X}$ lymphoblastoid cell line (LCL) that harbors the PCC phenotype from a patient homozygous for the S25*X MCPH1* mutation (5). MCPH1$^{S25X/S25X}$ cells should either entirely lack the MCPH1 protein (as the consequence of nonsense mediated decay) or only express a small truncated protein product containing residues 1–25 (1). However, surprisingly, radiation-induced MCPH1 foci formation was still observed in these patient cells (Fig. 1*A*). We speculated that there might be an alternative translational start site downstream of the S25*X* stop codon, which resulted in the deletion of the MCPH1 N-terminal region. Because this alternatively translated product would still contain the C-terminal tandem BRCT domains, it should localize normally to sites of DNA breaks based on previous studies (4–6). When we examined the MCPH1 sequence, we found a methionine residue just 10 residues downstream of the Ser-25 site. If this Met residue would be used as an alternative start codon, we would expect to see a form of MCPH1 that is only slightly smaller than WT MCPH1 in cells obtained from patient with S25*X* mutation.

To detect MCPH1 expression in patient cells (MCPH1$^{S25X/S25}$), we performed an IP-Western blot analysis using a WT lymphoblastoid cell line and a parental cell line (MCPH1$^{+/S25X}$) as controls. Interestingly, we found that the LCLs containing the

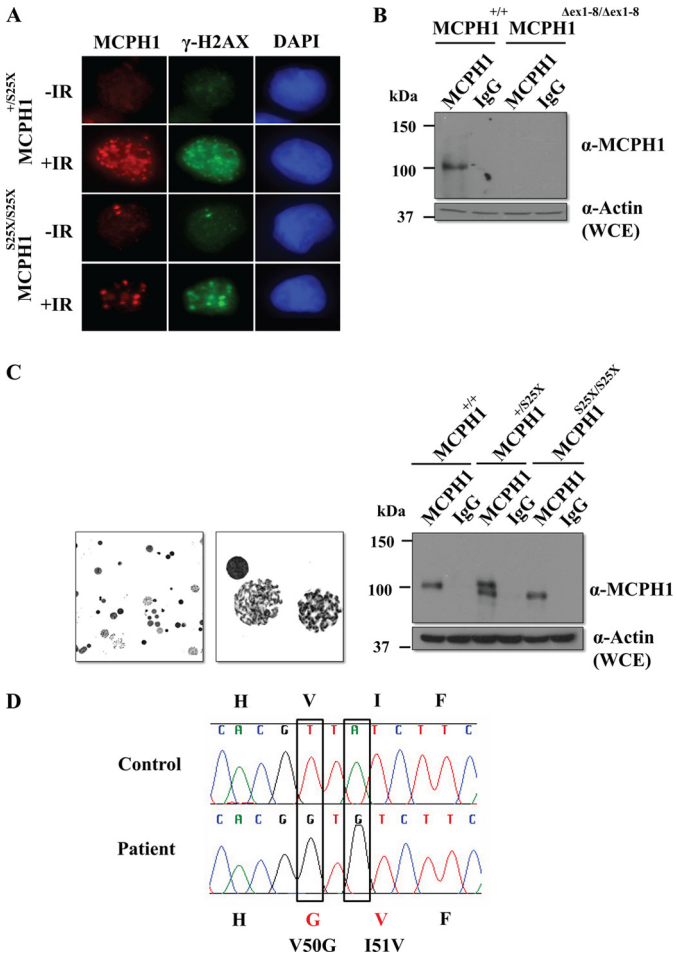

FIGURE 1. **MCPH1$^{S25X/S25X}$ cells express a truncated form of MCPH1 lacking its N terminus.** *A*, LCLs from patients with either heterozygous or homozygous S25*X* mutations were assayed for MCPH1 foci formation after ionizing radiation. After a 10 Gy ionizing radiation, cells were spun down onto coverslips and stained with the indicated antibodies. *B*, *upper panel*, the MCPH1 antibody specifically detects endogenous MCPH1 protein. Cell extracts prepared from WT cells and a primary microcephaly patient cell line (MCPH1$^{\Delta ex1-8/\Delta ex1-8}$) with a homozygous deletion of the promoter and exons 1–8, were immunoprecipitated and blotted with anti-MCPH1 antibodies. *Lower panel*, a truncated form of MCPH1 is detectable in patient cells with the S25*X* mutation. Cell extracts prepared from WT cells and cells from primary microcephaly patients were immunoprecipitated and blotted with anti-MCPH1 antibodies. Anti-actin immunoblotting of whole cell extract (WCE) was included as loading controls. *C*, cytogenetic analysis of peripheral blood lymphocytes from a primary microcephaly patient with the MCPH1$^{V50G,I51V/V50G,I51V}$ mutation, illustrating the PCC phenotype. *Left*, low power view. *Right*, high power view of prophase-like cells with high condensed chromosomes. *D*, sequence electropherogram of a primary microcephaly patient homozygous for c.149T>G and c.151A>G mutations.

heterozygous S25*X* mutation had two bands, whereas LCLs with homozygous S25*X* mutations only had one band, which migrated slightly faster than the predicted size of MCPH1 in WT LCLs (Fig. 1*B*). These observations are consistent with the postulated alternative translational start site for MCPH1 transcripts in MCPH1 S25*X* patient cells, and production of a truncated MCPH1 protein with a partial deletion of its N-terminal BRCT domain.

Disruption of the N-terminal BRCT domain is specifically associated with defective in chromosome condensation, consistent with our previous study (8). Further genetic findings also suggest a critical role for this domain both the PCC and micro-

cephaly phenotypes as we also identified missense mutations targeting this BRCT domain. The PCC phenotype was observed on routine karyotyping of a six-month-old child of non-consanguineous parents (Fig. 1*C*), with primary microcephaly (OFC$<0.4^{th}$ centile) and an antenatally detected cystic hygroma (data not shown). Given the association of this cytogenetic phenotype with mutations in the MCPH1 gene (9), capillary sequencing was performed on all 14 coding exons of the microcephalin gene. Homozygous mutations c.149T>G and c.151A>G were identified in consecutive codons, resulting in amino acids substitutions V50G and I51V (Fig. 1*D*). Both mutations alter conserved residues in the N-terminal BRCT domain and were not present in 300 controls.

Three other homozygous missense mutations have been reported in primary microcephaly patients (T27R, H49Q, S72L), and significantly, all of these cluster at the N terminus of MCPH1 (13, 14). All of these mutations are associated with the PCC phenotype, reinforcing the importance of this domain for disease causation and chromosome condensation.

SET Is an MCPH1-interacting Protein—Given the above result and our previous observation (8), we therefore sought to identify MCPH1-associated proteins that are involved in the regulation of chromosome condensation. We performed TAP specifically looking for proteins that may associate with the N terminus of MCPH1. After excluding the heat shock proteins and ribosomal proteins as general contaminants that usually interact with overexpressed proteins, we identified the SET nuclear oncogene as a potential binding partner of MCPH1 (Fig. 2*B*, *left panel*).

To confirm that MCPH1 interacts with SET, we transiently expressed SFB-tagged WT or deletion mutants of MCPH1 in 293T cells. We found that WT MCPH1 and an N-terminal fragment of MCPH1 (N-BRCT; residues 1–130) co-precipitated with endogenous SET but found that the N-terminal deletion mutant of MCPH1 (ΔN; residues 131–835) failed to do so (Fig. 2*B*, *right panel*). We also performed *in vitro* binding experiments using GST or GST-fusion proteins. GST-fused WT MCPH1 or an N-BRCT of MCPH1 interacted with SET *in vitro*; however, GST-fused ΔN MCPH1 did not show any appreciable binding to SET (Fig. 2*C*). The reverse pulldown assay using GST-fused SET also revealed that SET specifically associated with the N terminus of MCPH1 (Fig. 2*D*). Together, these data suggest that SET directly binds to the N terminus of MCPH1.

SET Down-regulation Leads to Aberrant Chromosome Condensation—Because the N terminus of MCPH1 is required to prevent PCC, we investigated whether the SET protein is involved in the same function. When we down-regulated SET expression in WT MEFs using siRNA specifically targeting murine SET, we observed the identical cellular phenotype to that characteristic of Mcph1$^{-/-}$ MEFs (Fig. 3*A*). About 10–15% of MEF cells with siRNA-mediated SET down-regulation exhibited a prophase-like PCC phenotype (Fig. 3*A*), and ~30% of metaphases had highly compact, condensed chromosomes (Fig. 3*B*). This result indicates that the SET protein may function together with MCPH1 in preventing premature chromosome condensation.

Similarly, in human cells, a significantly higher percentage of cells with highly compact, condensed chromosomes were observed among H1299 cells with SET knockdown than among those cells transfected with control siRNA (Fig. 3*C*), indicating that SET has a similar function in human cells.

Given that depletion of condensin II alleviates the chromosome condensation phenotype observed in cells derived from patient with MCPH1 mutations (10), we tested whether the abnormally condensed metaphases and the PCC phenotype observed after SET knockdown could also be alleviated by condensin II down-regulation. We co-depleted hCAP-D3, a subunit of the condensin II complex, with SET in H1299 cells and indeed observed a significantly lower percentage of cells with the PCC phenotype or highly condensed metaphase chromosomes than among H1299 cells with only SET down-regulation (Fig. 3*C*). Together, these data support our hypothesis that SET acts with MCPH1 and participates in the negative regulation of condensin II, which is important for regulation of chromosome condensation *in vivo*.

MCPH1 V50G/I51V Mutant Has a Reduced Binding Affinity to SET and Fails to Rescue Chromosome Condensation Defect—Because primary microcephaly-associated missense mutations localize at the N terminus of MCPH1, it would be interesting to test whether such MCPH1 mutations would affect the interaction between MCPH1 and SET. Therefore, we specifically examined the novel V50G/I51V mutations that we had identified. Co-precipitation and GST pulldown experiments showed that compared with that of WT MCPH1, the interaction between this mutant MCPH1 protein and SET was greatly reduced (Fig. 4, *A–C*). In addition, reconstitution of MCPH1 V50G/I51V into Mcph1$^{-/-}$ MEFs failed to fully rescue the PCC (Fig. 4*E*) and abnormal condensed metaphases phenotypes observed in these cells (Fig. 4*D*), indicating that the interaction between MCPH1 and SET is important for regulating chromosome condensation *in vivo*.

DISCUSSION

MCPH1 plays an important role in diverse cellular functions, including DNA repair, cell cycle control, and regulation of telomerase activity (6, 7, 10, 11, 13, 15, 16). The most prominent phenotype of MCPH1-deficient cells is increased number of prophase-like PCC cells and highly compacted metaphase chromosomes. However, exactly how MCPH1 regulates chromosome condensation remains largely unknown. Here, we demonstrated that the regulation of proper chromosome condensation carried out by the N-terminal domain of MCPH1, which binds directly to SET, identified in this study as an MCPH1-associated protein. Our results support that MCPH1 and SET function together and participate in the regulation of chromosome condensation.

The clinical phenotype of aberrant chromosomal condensation in patients with MCPH1 mutations is well characterized (9). However, to date, *MCPH1* patient cells do not appear to have increased sensitivity to DNA-damaging agents (17, 18), and increased tumor incidence has not been reported in primary microcephaly patients with *MCPH1* mutations. This suggests that *MCPH1* mutations in these patients do not impair the DNA damage repair function of MCPH1 (19).

On the basis of our results, we propose that the *MCPH1* S25*X* mutation identified in primary microcephaly disrupts only the

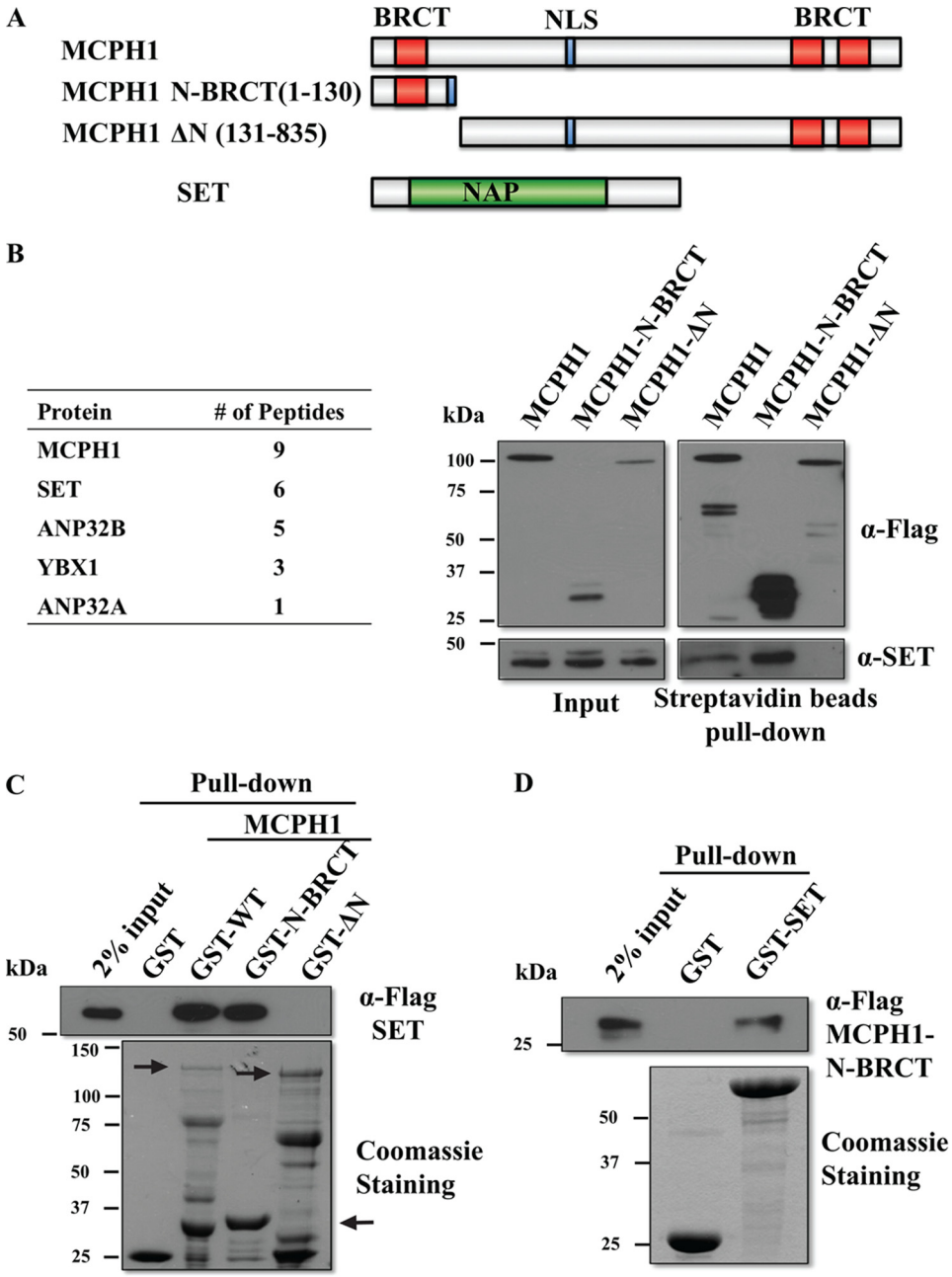

FIGURE 2. **SET is a novel binding partner of MCPH1.** *A*, schematic representation of WT MCPH1, MCPH1 deletion mutants, and SET nuclear oncogene. *B*, *left panel*, TAP of complexes containing MCPH1-N terminus revealed SET as an MCPH1-associated protein. 293T cells stably expressing an MCPH1 N-terminal fragment were used for TAP. MCPH1-associated proteins were identified by LC-MS/MS performed at the Taplin Mass Spectrometry Facility at Harvard University. *Right*, SFB-tagged WT MCPH1 and MCPH1 N-BRCT co-precipitated with endogenous SET, but the N-terminal deletion (ΔN) mutant of MCPH1 did not. *C*, GST-fused WT MCPH1 and MCPH1 N-BRCT, bound to SET *in vitro*, but the GST-fused MCPH1 ΔN or GST alone did not. *D*, GST-SET, but not GST alone, pulled down the MCPH1 N-BRCT. *Arrows* indicate the expected migration for each recombinant protein. *NLS*, nuclear localization signal; *NAP*, nucleosome assembly protein.

N-terminal BRCT domain of MCPH1. The alternative translation start site allows a truncated MCPH1 protein product to be made that may retain most, if not all, of its functions in DNA damage response, in the case of this mutation. However, this truncated protein, which lacking its N-terminal BRCT domain, cannot interact with SET. In addition to the S25*X* mutation, we also identified a novel double V50G/I51V mutation of *MCPH1* in a primary microcephaly patient. This V50G/I51V mutant is also defective in SET binding and failed to fully rescue the abnormal chromosome condensation phenotype. Together, with other published missense mutations in MCPH1 (13, 14), these data strongly suggest that microcephaly and PCC observed in patients with MCPH1 mutations arise mainly as a result of the disruption of the MCPH1 N-terminal BRCT domain and consequently the MCPH1-SET interaction.

We provide additional evidence to support a role of SET in chromosome condensation using complimentary experimental strategies. Knockdown of SET in both mouse and human cell lines resulted in a PCC phenotype. Similar to that of MCPH1-deficient cells, the depletion of hCAP-D3, a subunit of the con-

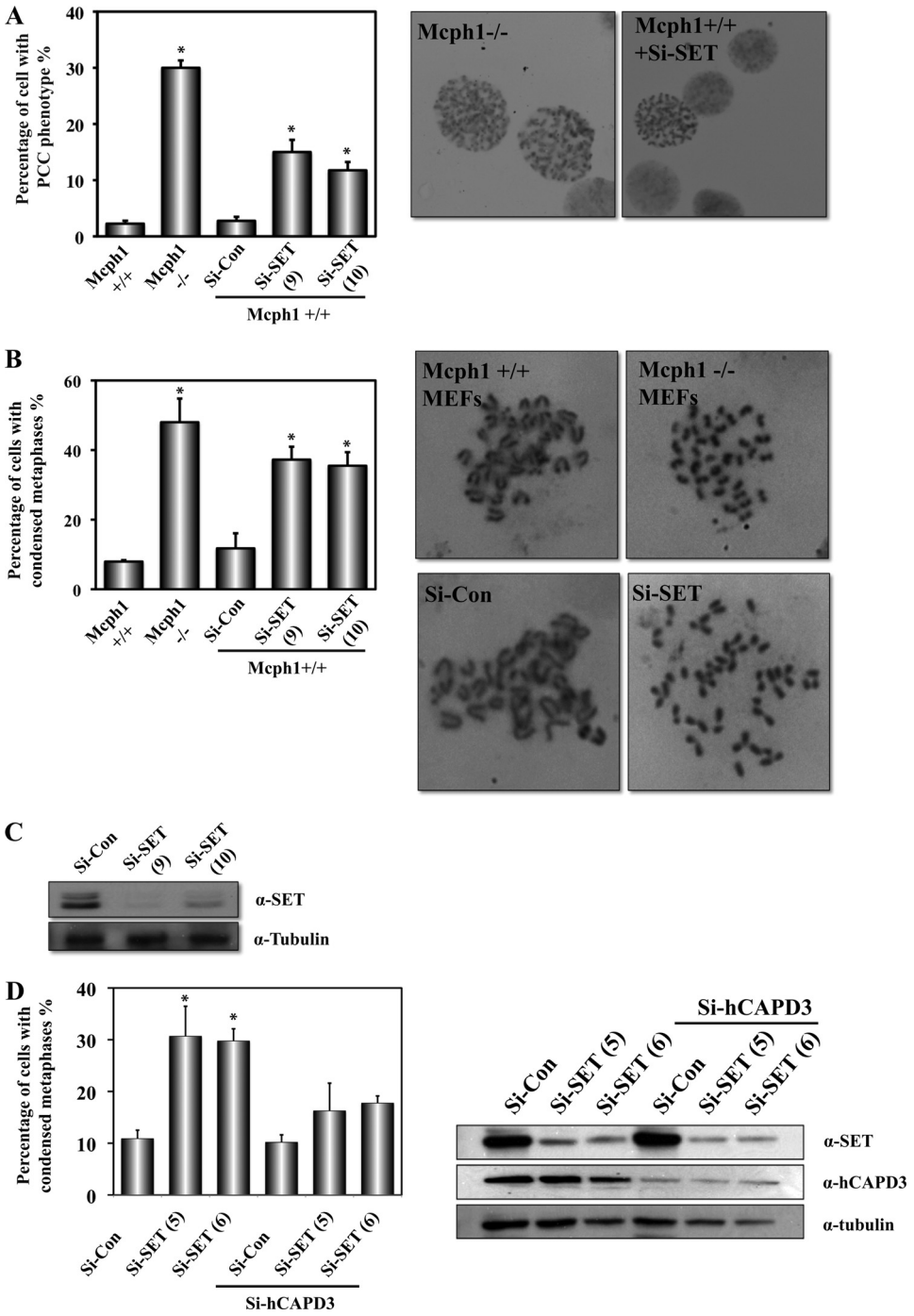

FIGURE 3. **SET deficiency leads to aberrant chromosome condensation.** *A*, Mcph1$^{-/-}$ MEFs and mouse fibroblasts depleted for SET by siRNA, displayed substantial numbers of prophase-like cells with condensed chromosomes. *B*, knockdown of SET using two different siRNA (9 and 10) in WT MEFs increased the percentage of cells with condensed metaphases compared with MEFs transfected with control siRNA. *C*, Western blot analysis confirmed the knockdown of SET in MEFs. *D*, knockdown of SET using two different siRNA (5 and 6) in human H1299 cells increased the percentage of cells with condensed metaphases compared with H1299 cells transfected with control siRNA. Co-depletion of hCAP-D3 partially rescued the percentage of H1299 cells with condensed metaphases in SET knockdown cells. Bar graphs are presented as means ± S.E. *, $p < 0.05$; relative to control, si-Con, Mann-Whitney *U* test.

densin II complex, partly reversed the PCC phenotype in SET knockdown cells. These data further validate that SET acts in the same pathway as MCPH1 and participates in the regulation of chromosome condensation.

Exactly how SET regulates chromosome condensation remains to be resolved. Previous studies suggested SET may possess chromatin decondensation activity at its acidic C terminus (20–22). In addition, the expression level of SET oscillates throughout the cell cycle and is markedly reduced in the G_2 phase, which coincides with Cdk1 activation (23). SET has also been suggested to be a negative regulator of mitotic entry by blocking cyclin B-CDK1 (24). SET also binds to histones, protecting them from acetylation by acetyltransferases, and this function may contribute to the potential role of SET in regulating chromatin compaction and transcription (25, 26). Whether any of these known functions or additional functions of SET are

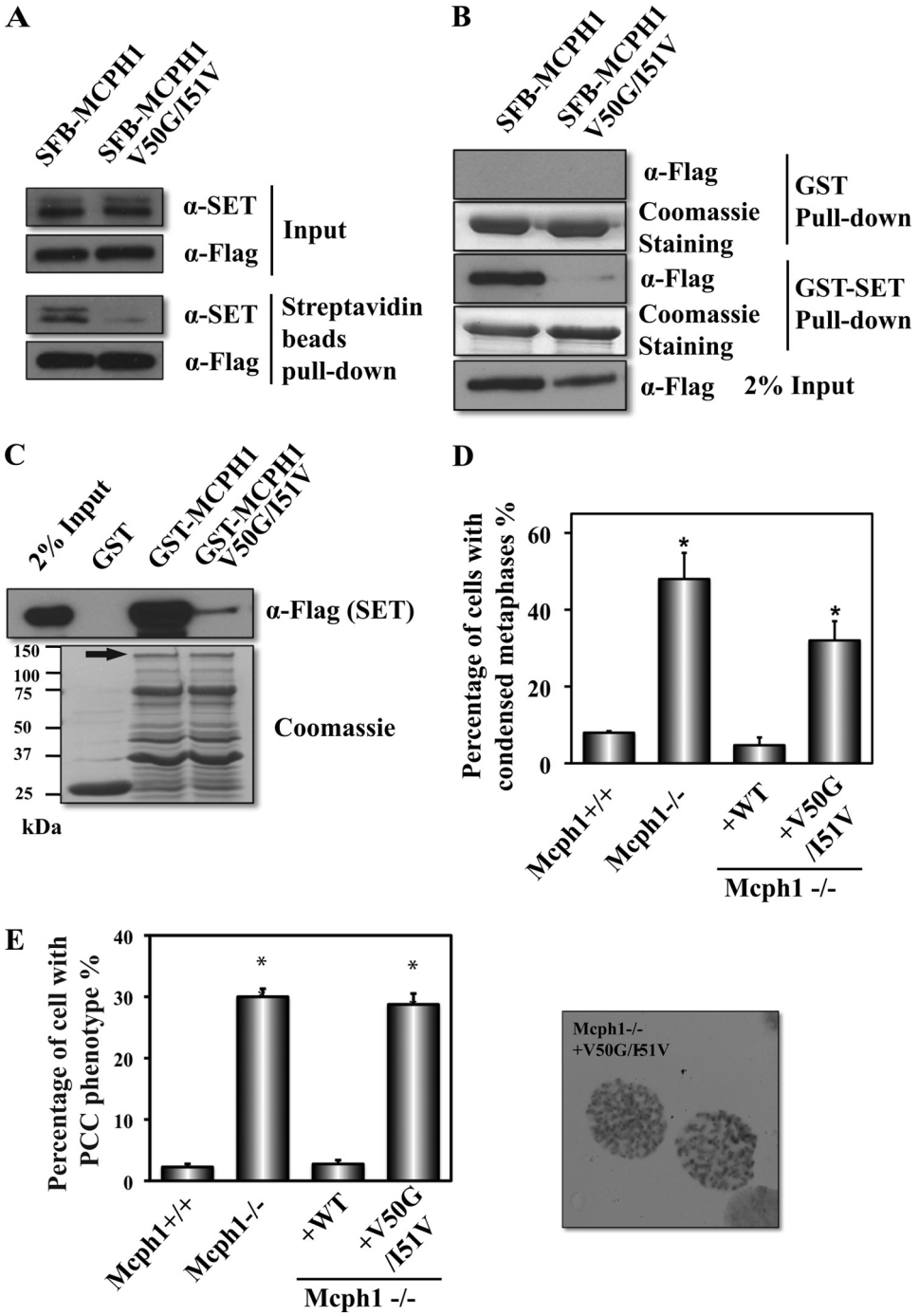

FIGURE 4. **MCPH1 V50G/I51V mutant protein has reduced affinity for SET and fails to fully rescue the chromosome condensation defect.** *A*, MCPH1 V50G/I51V mutations greatly reduced the binding of MCPH1 with SET. Lysates were prepared from 293T cells transfected with plasmids encoding SFB-tagged WT MCPH1 or MCPH1 V50G/I51V mutant. Precipitation was performed using streptavidin beads, and immunoblotting was conducted using antibodies as indicated. *B*, beads coated with GST or GST-SET were incubated with 293T cell lysates containing SFB-tagged WT MCPH1 or MCPH1 V50G/I51V mutant. After extensive washing, associated proteins were eluted and immunoblotted with anti-FLAG antibodies to detect epitope-tagged MCPH1. Coomassie staining was included to indicate the equal amounts of GST and GST-SET used in these experiments. *C*, reciprocal GST pulldown assay shows reduced *in vitro* binding between SET and MCPH1 V50G/I51V mutant. *Arrow* indicates the expected migration for these recombinant proteins. *D*, a MCPH1 V50G/I51V mutant reconstitution did not fully rescue the abnormal chromosome condensation phenotype in Mcph1$^{-/-}$ MEFs. *E*, a MCPH1 V50G/I51V did not rescue the PCC phenotype in Mcph1$^{-/-}$ MEFs. *, $p < 0.05$ with respect to Mcph1$^{+/+}$ cells; Mann-Whitney *U* test.

involved in the regulation of chromosome condensation awaits further investigation.

In summary, we reported the identification of SET as an MCPH1-associated protein and an important negative regulator of chromosome condensation. The results presented here support the model that MCPH1 regulates proper chromosome condensation via its N terminus, which interacts and functions with SET. Further studies are warranted to elucidate whether the two distinct functions of MCPH1, its involvement in DNA damage repair and its role in chromosome condensation, represent distinct cellular roles or indeed act together during key cellular processes.

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